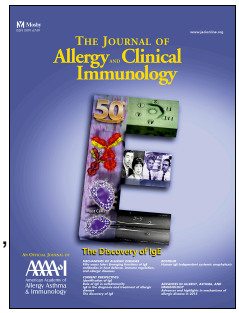
DOI:
10.1016/j.jaci.2017.11.010

Document Version
Peer reviewed version

Accepted Manuscript



Food Allergy: Update on Prevention and Tolerance

George Du Toit, M.B., B.Ch., Hugh Sampson, MD, Marshall Plaut, MD, Wesley Burks, MD, Cezmi Akdis, MD, Gideon Lack, M.B., B.Ch.

PII: S0091-6749(17)31815-8

DOI: 10.1016/j.jaci.2017.11.010

Reference: YMAI 13138

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 22 September 2017

Revised Date: 21 November 2017

Accepted Date: 22 November 2017

Please cite this article as: Du Toit G, Sampson H, Plaut M, Burks W, Akdis C, Lack G, Food Allergy: Update on Prevention and Tolerance, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.11.010.

Food Allergy: Update on Prevention and Tolerance

George Du Toit, M.B., B.Ch., Hugh Sampson, MD, Marshall Plaut, MD, Wesley Burks, MD, Cezmi Akdis, MD, Gideon Lack, M.B., B.Ch.

From the Department of Paediatric Allergy, Division of Asthma, Allergy and Lung Biology, King's College London and Guy's and St. Thomas' NHS Foundation Trust, London

Abstract

Of the many possible hypotheses which explain the recent rise in childhood food allergy, the dual allergen exposure hypothesis has been the most extensively investigated. This chapter serves as a review and update on the prevention of food allergy, and focuses on recently published Randomized Controlled Trials (RCTs) exploring the efficacy of oral tolerance induction in infancy for the prevention of food allergy. As a result of these RCTs, National Institutes of Health (NIH) recommendations now actively encourage the early introduction of peanut for the prevention of peanut allergy and other countries/settings recommend the inclusion of potential common food allergens including peanut and egg in complementary feeding regimens commencing at approximately 6 months of age, but not before 4 months.[1-3] Further studies which explore the efficacy of oral tolerance induction to other common food allergens, and which focus on optimal timing, duration and adherence are required.

Abbreviations

CI - Confidence interval
ITT - Intention-to-treat
PP – Per Protocol
SCORAD - SCORing Atopic Dermatitis
SPT - Skin Prick Test
LEAP Study - Learning Early About Peanut Allergy Study
LEAP-On Study - 12 month extension of LEAP Study: Persistence of Oral Tolerance to Peanut
EAT Study – Enquiring about Tolerance Study. (Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants)
HEAP - Hens' Egg Allergy Prevention
STAR - Solids Timing for Allergy Research
STEP - Starting Time for Egg Protein
BEAT -Beating Egg Allergy
PETIT - Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema

Key words: Food Allergy; Peanut Allergy; Egg Allergy; Allergy prevention.

Prevention of food allergy

'An ounce of prevention is worth a pound of cure' is an appropriate adage to describe much research into food allergy (FA) over the past decade. Given there is currently no cure, research has increasingly focused on interventions aimed at FA prevention. These interventions are generally applied early in life and include primary prevention, which seeks to prevent the onset of IgE sensitization and secondary prevention, which seeks to interrupt the development of FA in IgE-sensitized children.

This chapter will discuss possible reasons for the increase in food allergy, review current knowledge around methods for primary prevention from recently published research, describe statistical issues in FA prevention studies and briefly outline potential directions for future research. The main focus will be on lessons learned from the recently published LEAP (Learning Early about Peanut Allergy), LEAP-On (Persistence of Oral Tolerance to Peanut) and EAT (Enquiring about Tolerance) randomized controlled trials [4-6] but other published FA prevention research is also included.

Hypothesizing the Increase in Food Allergy

Various hypotheses have been put forward to explain the rise in food allergy. Integration of the vitamin D deficiency, hygiene, and dual-allergen exposure hypotheses (which is the focus of this chapter) are shown in Figure 1.

This paper focuses on the 'dual allergen exposure hypothesis' which suggests that allergic sensitization to food occurs through low-dose cutaneous sensitization, whilst early consumption of food protein induces oral tolerance.[7] This hypothesis was developed following publication of studies demonstrating a strong association between dietary exposure, eczema and the development of food allergy.

i) Studies demonstrating the role of cutaneous sensitization in FA

Animal and human observational and in-vitro studies demonstrate transcutaneous sensitization to food allergens through inflamed eczematous skin. In humans, the topical application of Arachis (peanut) oil onto eczematous skin during infancy was significantly associated with peanut allergy in eczematous children.[8] Environmental exposure to peanut during infancy (assessed by household peanut consumption) increased the risk of peanut allergy; however, if the infant had consumed peanut in the first year of life, they were protected against developing peanut allergy.[9] More recent studies found that eczema severity amplifies the risk of peanut sensitization and likely allergy resulting from exposure to peanut antigen in household dust.[10] A similar increase of peanut sensitization and allergy risk was seen in children with filaggrin loss-of-function mutations exposed to high levels of peanut allergens in the household dust.[11] This provides a good example of gene-environment interactions leading to the development of peanut allergy in young infants.

A cross-sectional study assessed the route of peanut exposure in the development of allergy and captured maternal peanut consumption during pregnancy, breast-feeding, and in the first year of life via a questionnaire. Household peanut consumption was also quantified. The median weekly household peanut consumption in the patients with PA was significantly increased (18.8 g)

compared with control subjects without allergy (6.9 g) and high-risk control subjects (1.9 g).[9] These findings suggest that high levels of environmental exposure to peanut during infancy can promote sensitization and support the hypothesis that peanut sensitization occurs due to environmental exposure.

ii) Studies demonstrating the role of tolerance induction in early childhood

An ecological study exploring the prevalence of peanut allergy in infants in Israel compared with infants in the UK, found a significantly higher rate in the UK (1.85% vs 0.17%, $p<0.001$).[12] This 10-fold increase in the prevalence of peanut allergy in UK children remained when confounding factors were accounted for. One explanation for this difference is that peanut was introduced at an earlier age and consumed in larger quantities in the Israeli infants; 7.1 grams (g) of peanut protein per month compared to no exposure (0 g) to peanut protein in children in the UK ($p<0.001$).

The dual allergen exposure hypothesis combines observational data exploring cutaneous sensitization and early tolerance induction, and proposes that the balance of exposures during the first year of life (depending on whether the initial exposure to peanut is through the skin or gut), primes the immune system to develop allergy or tolerance (respectively). A window of opportunity exists during the child's first year of life within which to influence a tolerogenic response. The dual allergen exposure hypothesis, predominantly under the guise of oral tolerance induction, has been tested in several RCTs which are discussed below.

Randomized Controlled Trials of Oral Tolerance Induction

For the purposes of this chapter we consider the use of the term 'tolerance' to be a state of clinical unresponsiveness to a known allergen. Later in this chapter we discuss the evidence that, after oral tolerance induction programs, tolerance may be enjoyed without the need for ongoing exposure to that allergen.

a. Peanut allergy

The LEAP study was developed following publication of observational data that early and regular consumption of peanuts was associated with the prevention of peanut allergy, particularly in children who are at a higher risk due to a compromised skin barrier.[11, 12] The LEAP study was a randomized controlled trial that assessed oral tolerance induction of peanut in high-risk children (severe eczema and/or egg allergic) aged between 4 and 11 months of age in the UK. Infants were randomized to consuming peanut products at least 3 times a week (average of 6 g of peanut protein a week) or completely avoiding any peanut until 60 months of age.[4] LEAP results showed that early introduction and regular on-going consumption of peanut resulted in a significant reduction in the number of children with peanut allergy at 60 months of age compared to those who avoided peanut. The intention-to-treat analysis showed that in the peanut avoidance group, 17.2% of the children had challenge-proven peanut allergy at 60 months of age compared with 3.2% in the peanut consumption group (81% relative reduction). Furthermore, the LEAP study demonstrated both primary and secondary prevention: there was a reduction in peanut allergy at 60 months of age in those children who had peanut introduced early, regardless of their sensitization status at baseline (based on skin prick test and specific-IgE levels). (**Figure 2)**

Early introduction of peanut was also found to be effective at preventing peanut allergy in a per protocol - but not the intention-to-treat (ITT) - analysis of children who participated in the EAT study, an RCT in which exclusively breastfed children from the general population were randomized to consume peanut (alongside 5 other allergenic foods) from 3 months of age or continue exclusive breastfeeding until approximately 6 months after which time parents introduced allergenic foods as they wished.[6] Children who introduced peanut from 3 months of age as per protocol were significantly less likely to develop peanut allergy than those who followed United Kingdom Department of Health advice to delay solid food introduction until approximately 6 months of age (Per Protocol analysis: 0% vs 2.5%, p=0.003). It is important to acknowledge that per-protocol analyses may introduce hidden bias unless the probability of receiving the intervention is random with respect to all predictors of a study's outcome. However, an instrumental variable analysis (IVA) of the EAT data showed no evidence that the per-protocol estimate of efficacy was biased,[13] suggesting that, even in the general population, early introduction of peanut is an effective prevention strategy.

In a recently published meta-analysis of oral tolerance induction, Lerokiadonou *et al*. note 'moderate certainty' of evidence that introducing peanut between 4 and 11 months reduced the risk of developing peanut allergy (RR 0.29; 95%CI 0.11–0.74) based on two RCTs (LEAP and EAT) investigating early peanut introduction in 1550 children.[14]

b. Egg allergy

Six RCTs from different countries have now published their findings assessing introduction of egg during infancy for the prevention of egg allergy, detailed in Table 1. There is great variability in the populations enrolled (high risk vs. population cohorts) and in the form of egg used in these studies (ranging from pasteurized raw whole egg to less allergenic extensively heated egg) which makes it difficult to compare the findings. Nonetheless there are some commonalities between the outcomes of the studies.

Two RCT's made use of egg sensitization as the primary study outcome; whilst no significant effect in egg white specific IgE was noted in HEAP, the BEAT study showed a significant difference between groups for egg white SPT.[15, 16] Four RCT's assess egg allergy by oral food challenge. No significant difference was noted in the STEP[17] or STAR studies (but recruitment was discontinued early in STAR).[18] The EAT study found a significant difference in egg allergy, this was only true for the per protocol population.[6] The PETIT study is the only RCT to demonstrate a statistically significant lowering of allergy to egg in the ITT analyses.[19] In PETIT, infants with eczema at age 4–5 months (n = 147) were recruited and assigned to either the placebo or intervention group. Uniquely, this trial made use of heated egg powder and extremely low starting doses (25 mg of egg protein, equivalent to 0·2 g of whole egg boiled for 15 min). At completion, the prevalence of egg allergy (as determined by Oral Food Challenge (OFC) to a cumulative dose of 7 g of heated whole egg powder) was significantly lower in the intervention group compared to the control group (8 and 38%, respectively, RR 0.22, 95% CI 0.09–0.54, p = 0.0001). This interim finding prompted an early cessation in enrolment as per the study stopping rules. As many of the participants were egg sensitized at baseline, it may well be that this study reflects secondary, as opposed to primary prevention of egg allergy.

Whilst individual studies may show conflicting or inconclusive results, a meta-analysis by Lerokiadonou *et al*. found 'moderate certainty' of evidence that introducing egg between 4 and 6 months reduced the risk of developing egg allergy (RR 0.56; 95%CI 0.36–0.87) based on five RCTs including 1915 children.[14]

c. Other foods – EAT study

The EAT study examined oral tolerance through early introduction of six allergenic foods in over 1000 exclusively breastfed children.[6] In addition to egg and peanut (discussed earlier) the intervention group had cow's milk, wheat, sesame and fish introduced into their diets from 3 months of age. The control group followed standard UK government advice of exclusively breastfeeding until introduction of solid food at approximately 6 months of age. The randomized sequence of food introductions for the early introduction group was cow's milk (yoghurt) first, followed by peanut, egg, sesame and whitefish in random order with wheat introduced last. The main outcome was a challenge-proven diagnosis of allergy to one or more of the six foods at 1 year and 3 years of age. In the intention-to-treat (ITT) analysis, 7.1% of the infants in the standard group developed food allergy to one or more of the six potentially allergenic foods compared with 5.6% in the intervention group (not statistically significant p=0.32). However, in the per-protocol analysis, the prevalence of any food allergy was significantly lower in the early-introduction group compared to the standard-introduction group (2.4% vs 7.3%, p=0.01). The risk of having a positive skin prick test (SPT) to any food was 22% lower in the early introduction group compared to the standard introduction group at 12 months of age (p=0.07) and at 36 months of age (p=0.47). (Primary Outcome Data of the EAT Study is shown in Figure 3).

In conclusion, RCTs of oral tolerance induction to a range of foods have shown variable results. Nonetheless, the finding of 'moderate certainty" in the meta-analysis, Lerokiadonou *et al*. for the introduction between 4 and 11 months of age for peanut and hens egg is reassuring. [14] Their findings for fish and early introduction of milk or hydrolyzed formula were of 'low certainty' and 'no evidence' respectively.

Importantly, both LEAP and EAT demonstrated that the early introduction of allergenic foods into the infant's diet was achievable and safe and that this did not affect breastfeeding rates or later nutrition and growth. However, in all studies, adverse event data show that children experienced allergic reactions during the initial baseline food challenge and thus, especially in high risk populations, children may have pre-existing food allergy despite never having knowingly consumed the food. This is discussed further in the following section exploring 'windows of opportunity' for oral tolerance induction.

Concept of different windows of exposure possibly relating to different foods; age and immunological markers

Food allergy typically has its genesis early in infancy, and whilst the age of onset of different food allergies is variable, the body of evidence suggests that the pathogenesis of common food

allergies starts early in life. Several RCTs examining oral tolerance induction found infants to have a high level sensitization or be allergic to the food at baseline and, importantly, before any known oral exposure to the food.[15, 18] Thus, to maximize the effectiveness of oral tolerance induction, it is important to understand the age at which oral tolerance induction programs should be commenced.

At inclusion to the EAT study, 5.1% (33/652) of the early introduction group had a positive SPT to one of the six allergenic foods being introduced. EAT infants were all enrolled at 3 months of age, highlighting that sensitization to foods can begin in very early infancy.[6] In the LEAP study, 76 of the 899 patients screened were excluded from enrolment as they had an SPT of >4mm at between 4 and 11 months of age.[20] This group was older than those participants who were eligible for enrolment to LEAP and who had negative SPT at time of screening (8.3 months of age (SD1.88) vs 7.7 months of age (SD 1.74)) and the median peanut SPT wheal diameter in this group was suggestive of peanut allergy at 7.5mm (IQR 6.0-9.0).[20] Data from LEAP and EAT thus suggest that, for oral tolerance to be effective, it should be commenced early, when high level sensitization is less likely to have occurred. To this end recently published allergy prevention recommendations suggest that introduction is targeted to early infancy but not before 4 months of age.[1-3] However, as demonstrated in EAT, early life dietary interventions present logistical challenges as weaning must be balanced with infants' developmental ability to consume solid food. Further studies exploring the effect of age on food allergic sensitization and the efficacy of oral tolerance induction in very young infants are needed.

Whilst the evidence suggests that oral tolerance induction may be most effective in very young infants who are not yet sensitized to foods, it is also important to understand whether oral tolerance induction is effective in children who are already sensitized either because they did not introduce allergenic foods in early infancy, or because they became sensitized very early in life. The LEAP study excluded children with a skin prick test (SPT) >4mm. This *a priori* decision was based on the assumption that such children would be very likely peanut allergic. Whilst including children with larger skin prick tests I n the study would have been scientifically useful, several other studies have shown that using a greater than 4mm cut off as a surrogate marker for existing peanut allergy is reasonable regardless of the age or risk profile of the child. In the HealthNuts Study around 80% (95% CI, 73.0-87.4) of high-risk infants with an SPT wheal size of greater than 4 mm had challenge-confirmed peanut allergy as 12 months of age and the Basophil Activation Validation (BAT) Study, found the optimal cut-off for the diagnosis of peanut allergy in a UK cohort was greater than 4mm. [21 22]
There is a clear need for robust scientific data assessing the outcome of oral tolerance induction in infants who are sensitized (particularly high level sensitized) to food allergens. However, until these data are available, current studies suggest that 4mm is an appropriate cut off for clinical use.

Issues of dosage

In addition to the window of exposure, the efficacy of oral tolerance induction appears to be influenced by the dose of food used. There seems to be a critical level of protein consumption required for the development of oral tolerance. In a murine model, a single high dose of peanut flour (100 mg) promoted oral tolerance and prevented subsequent IgE sensitization and T-cell

proliferation. There is however a paucity of data in the human population as to the optimal dosage of an allergenic food protein for the development of long-lived oral tolerance. The LEAP Study peanut consumption recommendations were based on the upper quartiles of those noted for Israeli infants who appeared to be protected against peanut allergy. [12] In LEAP a dose of 6g of peanut protein per week was recommended and on average consumption of 7.1g of peanut protein per week was achieved. This LEAP intervention achieved an overall 81% reduction in the level of peanut allergy. As adherence was excellent in the LEAP study, it was not possible to explore a dose-response relationship but this was explored in the EAT study, where 31.9% of the early introduction group were able to adhere, and within those who were non-adherent, the level of food-specific adherence in the early consumption group was variable. It is of interest that the statistically derived protective level for oral tolerance to peanut in the EAT study mirrors the median consumption of peanut protein per week in the Israeli population (1.7g in Israel): statistical modeling of EAT data showed that approximately 2g of food protein per week protected against both peanut allergy and egg white allergy, reducing the burden of allergic disease by approximately 90%. This was also true for protection against developing positive SPT to egg white (including SPT to raw egg white). Dose Response Modeling is shown in Figure 4.

Low-level allergen exposure (to select aeroallergens) results in allergic responses whereas high-level allergen exposure drives tolerance.[23, 24] Current data suggest that gram rather than milligram doses of food protein will be required for oral tolerance induction, but studies which explore the effect of oral tolerance induction with differing doses are required. This is especially true in the context of prevention of multiple food allergies where, as seen in EAT, high dose consumption of multiple foods may present logistical problems.

Persistence of Oral Tolerance Induction

Whilst oral tolerance induction has been shown to be effective in preventing food allergy in the immediate term, claiming that tolerance, rather than a delay to onset of food allergy, has been achieved requires examination of the effects of avoidance of the food under investigation, and/or of *ad libitum* consumption. To date the only food allergy prevention study to address this question is the LEAP-On study which examined whether early consumption of peanut had a sustained effect on peanut allergy prevention after 12 months of peanut avoidance.[5] A total of 556 participants (88.5% (556/628)) from the original LEAP trial were enrolled in the follow-on study. The rate of adherence to avoidance was 90.4% in the peanut-avoidance group and 69.3% in the peanut-consumption group. At 72 months of age, peanut allergy remained significantly higher in the peanut-avoidance group compared to the peanut-consumption group, 18.6% vs 4.8% ($p<0.001$) respectively. The LEAP-On Study showed that the non-allergic status of children who had been consuming peanut remained stable over 12 months of subsequent peanut avoidance. Thus, the key finding of the two LEAP studies is that early introduction and consumption of peanut until 60 months of age causes a reduction in peanut allergy that persists at 72 months of age after a 12-month period of avoidance. Follow-on studies of the LEAP and EAT cohorts are underway to observe whether the effects of early tolerance continue to persist approximately seven years after the interventions were stopped and after *ad libitum* consumption. Future studies of oral tolerance induction should include long term follow up after *ad libitum* consumption into their design.

Factors affecting adherence

A greater understanding of the many factors that influence adherence are of great clinical and public health importance. The lower rate of adherence in the EAT study varied between foods; egg ingestion was lower than peanut and milk consumption, but higher than sesame, fish, and wheat (which was always the last of the foods to be introduced).[6] However, partial adherence among early-introduction group participants was not associated with any significant increase in allergy prevalence. This offers reassurance that children who are unable to comply with the intervention will not increase their risk of food allergy.
The LEAP study achieved a high adherence rate in the peanut introduction group (92%), however frequent contact between study personnel and participating families was built into the protocol and peanut introduction was successfully achieved in the LEAP consumption group after only a few study contacts.
There are many other reasons for the differences in adherence rates between LEAP and EAT including factors relating to the food's introduction regimens, and maternal and family factors such as education, cultural and ethnic differences. In EAT there was a marked influence of race on food allergy rates, being much higher in non-white participants with a stepwise increase from white (5.3%), to mixed ethnicity (9.4%), to Asian/black/Chinese participants (19.3%), $p<0.0005$. Conversely, there was a statistically significant stepwise reduction in adherence most notable in the early-introduction group with only one in seven Asian/black/Chinese participants adhering to the protocol ($p=0.01$).

Food-allergic children are typically allergic to more than one food however, single allergen oral tolerance induction appears to be allergen-specific i.e. early consumption of peanut had no effect on development or resolution of other food allergies or atopic diseases.[25] Thus, if food allergy prevention is to be achieved through early exposure, studies which explore the many factors that influence adherence are required so as to maximize the effect of the intervention by promoting and facilitating successful introduction of multiple foods in infancy.

Immunological changes in food allergy prevention

Oral tolerance induction has proven to be successful in achieving clinical tolerance to specific foods, suggesting that the dual allergen exposure hypothesis is an accurate representation of one of the mechanisms by which food allergy develops. As well as clinical tolerance the LEAP and LEAP-On studies have demonstrated immunological changes suggestive of immune tolerance. As is now discussed, the dynamics of change are unique to each immune marker.

i) Changes in peanut- SPT, and IgE against peanut and r Ara h 2

In the LEAP study, the mean SPT wheal size was smaller at 60 months of age in the consumption group compared with the avoidance group and remained smaller at 72 months of age in LEAP-On. In contrast, there was no difference in mean levels of IgE to peanut between groups throughout the LEAP study but differences were noted at 72 months of age in LEAP-On (lower in the LEAP peanut-consuming population). The mean levels of Ara h 2–specific IgE declined significantly in the peanut-consumption group from 30 months to 60 months during LEAP ($P<0.001$) and remained low at 72 months of age in LEAP-On. The inhibition of IgE

synthesis is further reflected by the fact that relatively few participants in the peanut-consumption group had high-level IgE to peanut and to Ara h 2 at 30, 60 and 72 months of age. Children who were allergic to peanut at 60 months of age already had higher peanut-specific IgE at 12 months, differences remained at 30 and 60 months of age. These findings suggest that the elaboration of IgE antibodies to foods occurs early in infancy and may take a very long time to switch off, likely due to the presence of long-lived memory B and plasma cells committed to IgE production.

ii) Peanut-specific IgG4 and IgG4/IgE changes

Peanut-specific IgG and IgG4 levels increased over time in both LEAP groups, however the peanut consumption group, who were largely protected against developing peanut allergy, had a significantly greater and earlier increase, which was already evident by 12 months of age. The overall balance between peanut-specific IgG4 and peanut-specific IgE reflected the participants' allergic status to peanut. In the LEAP-On study, peanut-specific IgG4 levels and peanut-specific IgG4/IgE ratios continued to be higher in the previous peanut consumption group than in the previous peanut avoidance group. However, IgG4 levels started to slowly drift down after 30 months, even in the peanut consumption group. In the participants who became allergic in the LEAP-On study (1.1% of the peanut consumption group and 1.1% of the peanut avoidance group), the ratio of peanut-specific IgG4/IgE declined between 60 and 72 months. Children from the peanut consumption group who were able to tolerate peanut continued to have low levels of peanut-specific IgE and high ratios of IgG4/IgE at 60 months in LEAP and this was maintained at 72 months. These observations indicate that IgG4 is associated with protection against the development of allergy. Peanut-specific IgG4 has recently been shown to inhibit basophil activation in vitro in response to peanut. **(Figure 5)**

Special statistical considerations relating to prevention studies in food allergy

There are critical issues in the design and statistical analyses of prevention studies that differ fundamentally from treatment studies.[13] For example, in treatment studies all subjects start with the disease and few will be cured due to the intervention. In prevention studies all subjects start without the disease and, even in high-risk studies such as LEAP, less than 20% will end up with the disease. This has two important consequences both with respect to data imputation and with respect to analysing changes in biomarkers of prevention. In treatment studies, an intention to treat analysis may impute an allergic outcome to missing data since this is the most likely outcome where allergy is assumed to persist unless there is evidence of benefit. However, imputing an allergic outcome to all children with missing data in a prevention study could likely obscure and severely bias the treatment effect; especially, if the dropout rate is comparable to or higher than the disease rate in the population. This difference in prevention studies also affects the interpretation of biomarker data. If only a small subgroup of subjects (e.g. 20%) are destined to develop the disease, then the immunological effects of a successful intervention may only be apparent in this subgroup of 20%. The absence of relevant biomarker changes in the 80% who are not destined to develop the disease in the intervention group may obscure or dilute biomarker differences between the intervention and controls groups. These problems may be overcome using statistical methodologies to control for bias, as recently detailed by H.T. Bahnson *et al.*[13]

Summary:

Of the many possible hypotheses which explain the recent rise in childhood food allergy, the dual allergen exposure hypothesis has been the most extensively investigated. Recently published RCTs provide evidence that introduction of peanut (and likely hen's egg white) in early infancy offers a successful strategy for the prevention of food allergy. NIH recommendations now actively encourage the early introduction of peanut for the prevention of peanut allergy and other countries/settings recommend the inclusion of potential common food allergens such as peanut and egg in complementary feeding regimens commencing at around approximately 6 months of age, but not before 4 months of age. Further studies which explore the efficacy of oral tolerance induction to other common food allergens, and which focus on optimal timing, duration and adherence are required.

Acknowledgements
We thank Henry T Bahnson, Helen Fisher, and Poling Lau for support in the preparation of this manuscript.

Figures & Tables:

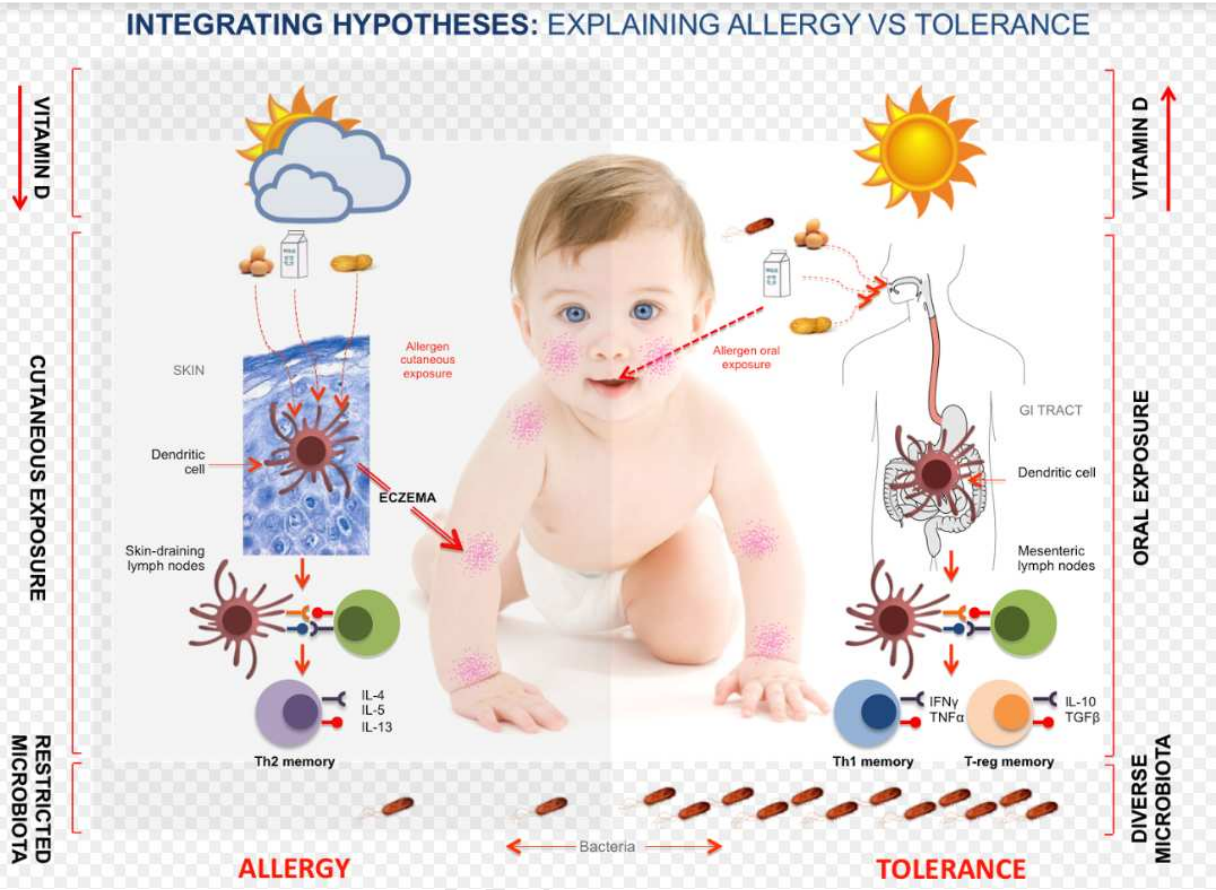


Fig 1. Integration of the vitamin D deficiency, hygiene, and dual-allergen exposure hypotheses. Sufficient levels of vitamin D, a diverse microbiota, and oral allergen exposure collectively support the development of tolerance. Conversely, allergic sensitization is promoted through cutaneous exposure, reduced diversity of microbiota, and vitamin D deficiency. Diminished microbial diversity and vitamin D deficiency are thought to interrupt the regulatory mechanisms of oral tolerance, with the latter also contributing to decreased epidermal barrier function. GI, Gastrointestinal; T-reg, regulatory T cells. Graphic modified from Lack G. Epidemiologic risks for food allergy. J Allergy Clin Immunol 2008;121:1331-6. Copyright © 2008 Elsevier. Reprinted with permission.

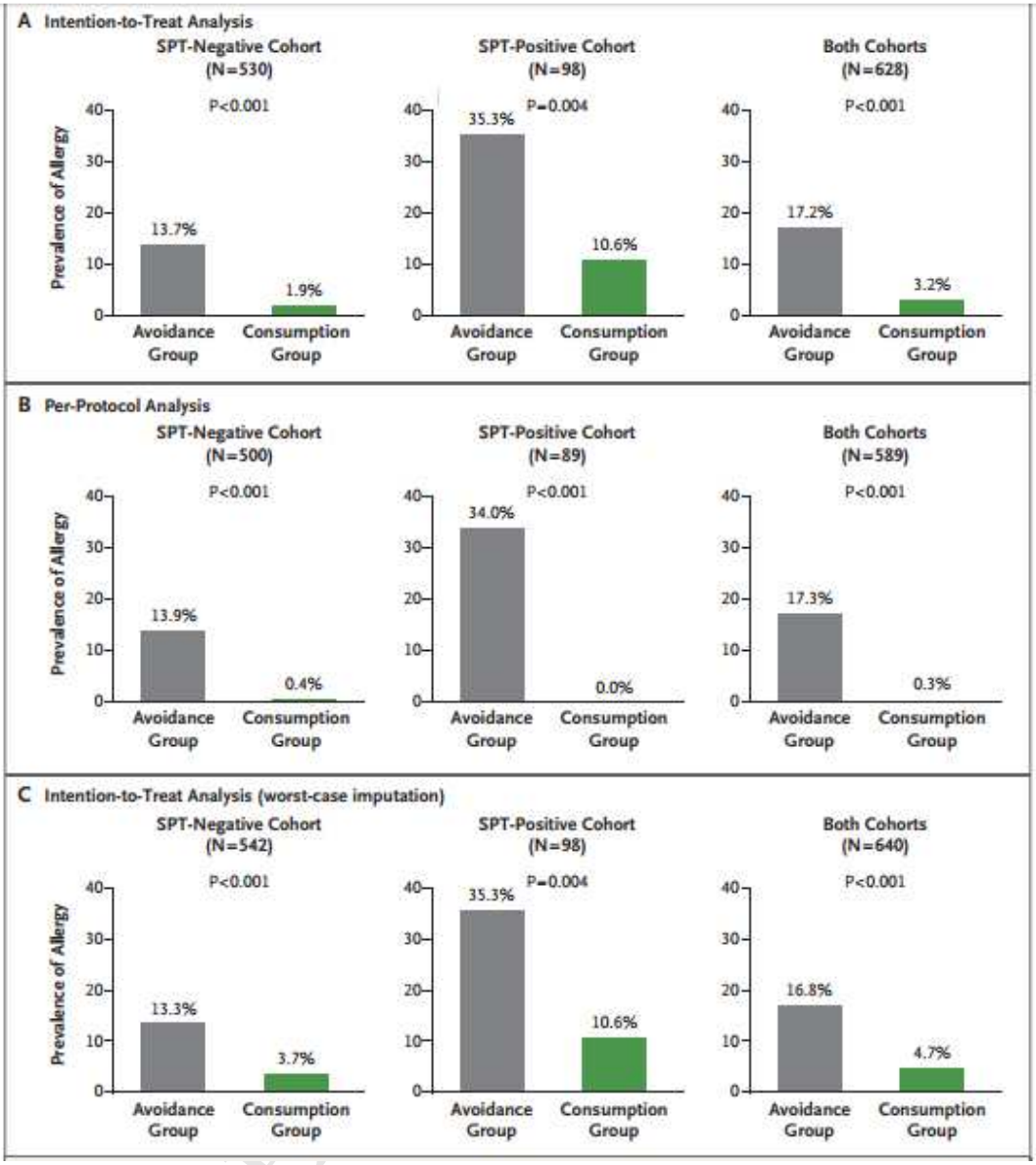


Figure 2. LEAP Study Primary Outcome findings (From Du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med 2015; 372:803-13. Copyright © 2015 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society)

The prevalence of peanut allergy at 60 months of age is shown among participants who had a negative result on the skin-prick test at baseline, among those who had a positive result at baseline, and in both groups combined, in the intention-to-treat analysis (Panel A) and the per-protocol analysis (Panel B). Among the 640 participants who underwent randomization, peanut-allergy status was determined by means of an oral food challenge in 617 (96.4%) and by means of a diagnostic algorithm in 11 (1.7%). Peanut allergy could not be evaluated with the use of the diagnostic algorithm in 2 participants (0.3%). A total of 10 participants (1.6%) voluntarily

withdrew or were lost to follow-up. The worst-case imputation analysis (Panel C) assumes that participants with missing data in the peanut-consumption group would have been allergic to peanuts and that participants with missing data in the peanut-avoidance group would have been nonallergic. P values are based on chi-square analyses.

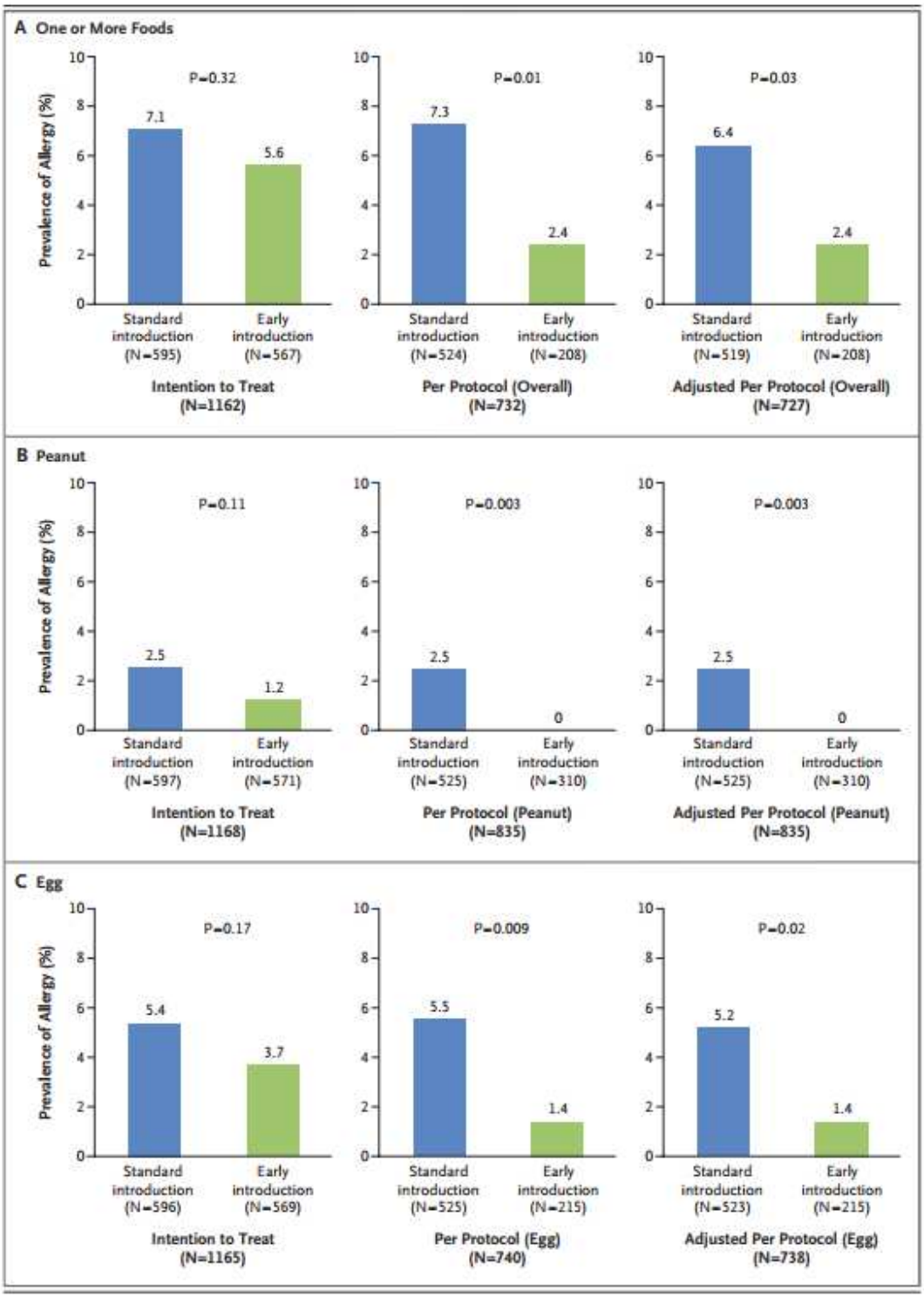


Figure 3. EAT Study Outcome findings (Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. N

The prevalence of IgE-mediated food allergy is shown with respect to one or more of the six early-intervention foods (peanut, cooked egg, cow's milk, sesame, whitefish, and wheat; Panel A), to peanut (Panel B), and to egg (Panel C). The first column shows the intention-to-treat analysis, the second column the per-protocol analysis, and the third column an adjusted per-protocol analysis. The intention-to-treat analysis included all the participants who had data that could be evaluated; the per-protocol population included all participants who adhered adequately to the assigned regimen. The adjusted per-protocol analysis was a conservative per-protocol analysis that adjusted the prevalence of food allergy in the standard-introduction group by subtracting the number of participants in the early-introduction group who had a positive result on the challenge at enrollment and who completed the trial with a confirmed food allergy from both the numerator (the number of participants with allergy in the standard-introduction group) and the denominator (the number of participants in the standard introduction group who adhered to the protocol). P values are based on chi-square analyses or Fisher's exact test, as appropriate.

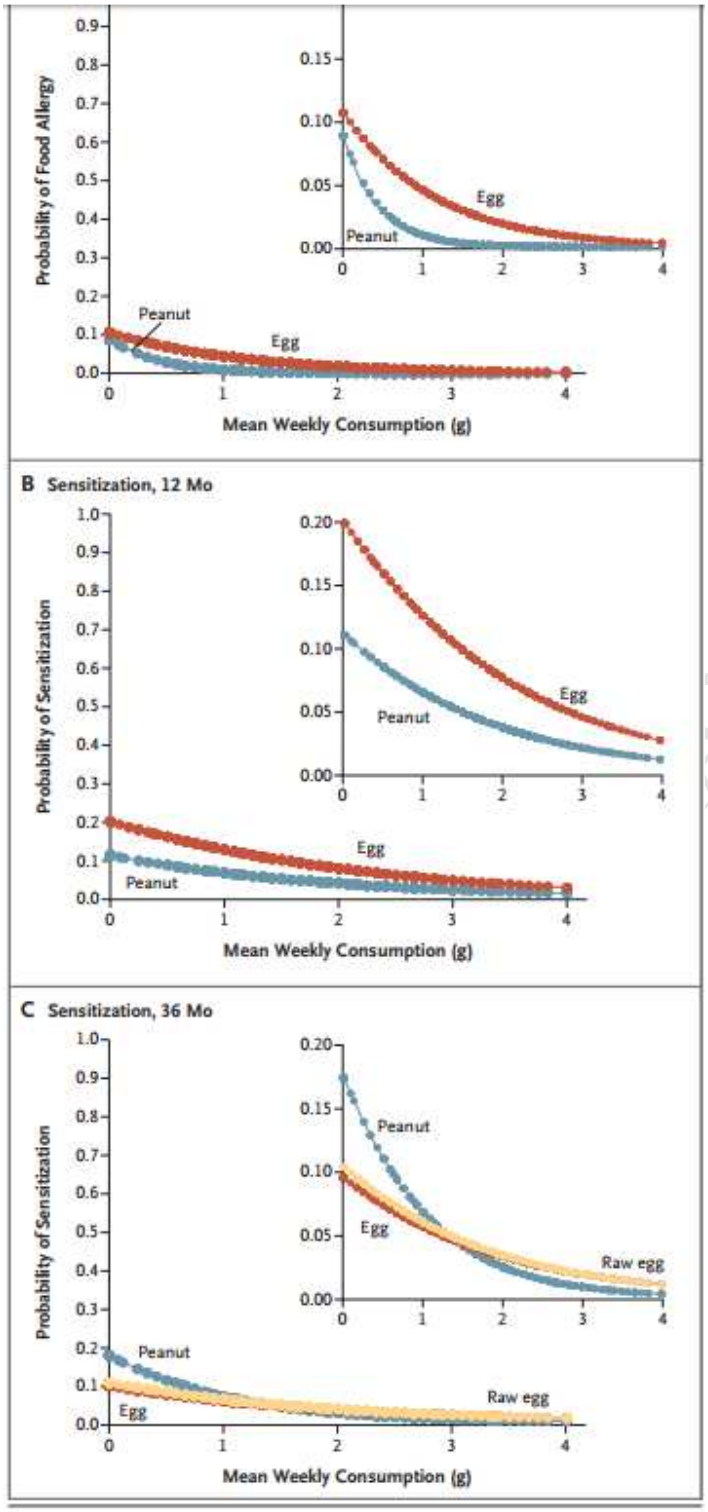


Figure 4. EAT Dose–Response Analysis of the Relationship between Mean Weekly Dose of Peanut or Egg Protein Consumed and Allergy or Positive Result on Skin-Prick Testing to Peanut, Egg, and Raw Egg White (Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. N Engl J Med

Shown are the predictive probability plots that were generated from statistical models of the prevalence of peanut allergy and egg allergy (Panel A) and of a positive result on skin-prick testing to peanut and egg at 12 months (Panel B) and to peanut, egg, and raw egg white at 36 months (Panel C), according to the mean weekly consumption of peanut and egg protein between enrollment and 6 months of age. The prevalence of both food allergy and positive skin-prick test diminishes with increasing levels of mean weekly consumption. Insets show the same data on an enlarged y axis

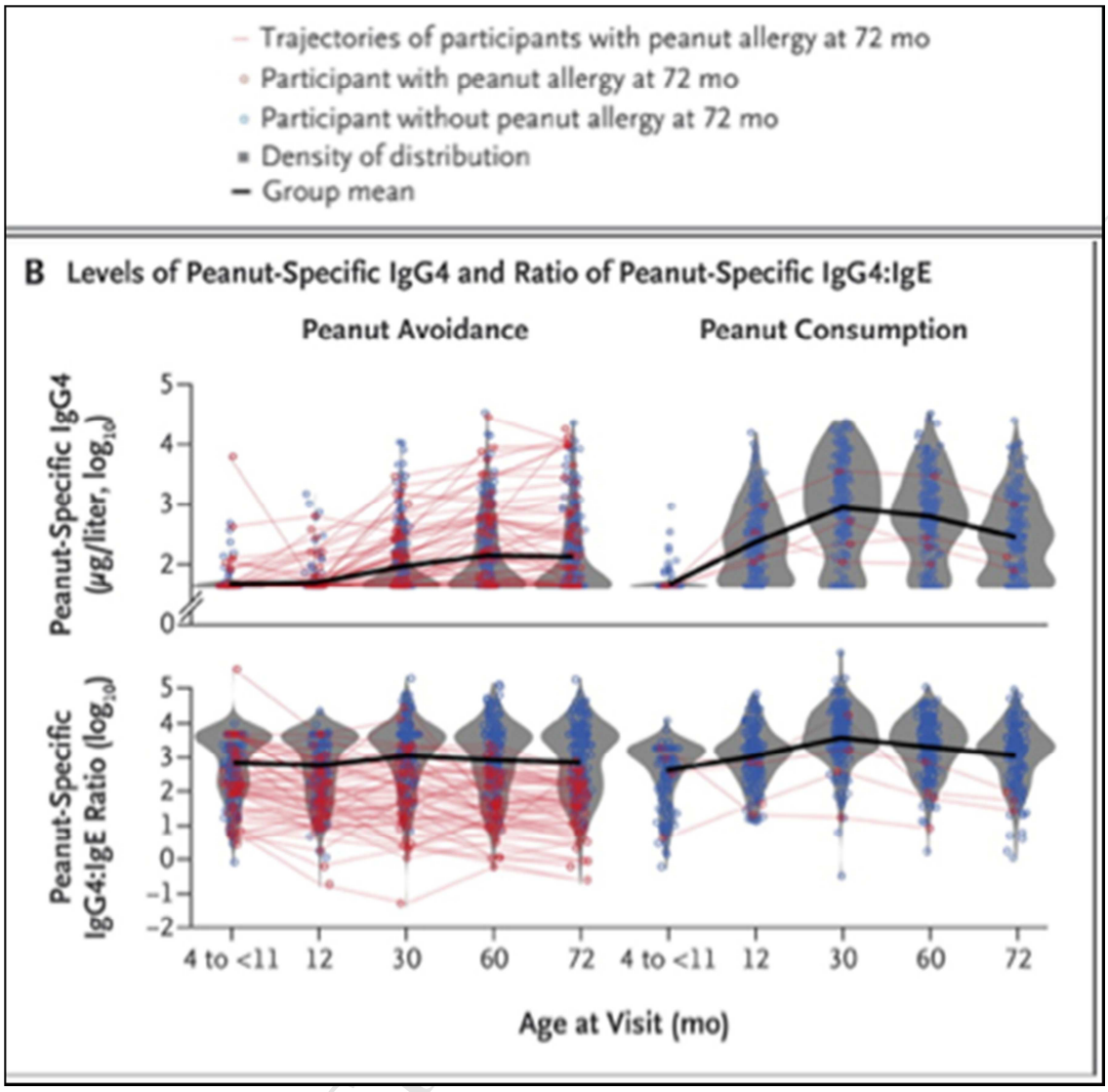


Figure 5. Changes that occur with IgE, IgG and IgE:IgG4 ratios over time ii children who consumed or avoided peanuts in the frame of the LEAP and LEAP-On studies (Du Toit G, Sayre PH, Roberts G, Sever ML, Lawson K, Bahnson HT, et al. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption. N Engl J Med 2016; 374:1435-43. Copyright © 2016 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society)

References:

1. Togias A, Cooper SF, Acebal ML, Assa'ad A, Baker JR, Jr., Beck LA, et al. Addendum guidelines for the prevention of peanut allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-sponsored expert panel. J Allergy Clin Immunol 2017; 139:29-44.
2. Netting MJ, Campbell DE, Koplin JJ, Beck KM, McWilliam V, Dharmage SC, et al. An Australian Consensus on Infant Feeding Guidelines to Prevent Food Allergy: Outcomes From the Australian Infant Feeding Summit. J Allergy Clin Immunol Pract 2017.
3. Joint SACN/COT Working Group on the timing of introduction of allergenic foods into the infant diet. 2017.] Available from https://cot.food.gov.uk/committee/committee-on-toxicity/cotwg/joint-sacn/cot-working-group-on-the-timing-of-introduction-of-allergenic-foods-into-the-infant-diet.
4. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med 2015; 372:803-13.
5. Du Toit G, Sayre PH, Roberts G, Sever ML, Lawson K, Bahnson HT, et al. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption. N Engl J Med 2016; 374:1435-43.
6. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. N Engl J Med 2016.
7. Lack G. Epidemiologic risks for food allergy. J Allergy Clin Immunol 2008; 121:1331-6.
8. Lack G, Fox D, Northstone K, Golding J, Avon Longitudinal Study of P, Children Study T. Factors associated with the development of peanut allergy in childhood. N Engl J Med 2003; 348:977-85.
9. Fox AT, Sasieni P, du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. J Allergy Clin Immunol 2009; 123:417-23.
10. Brough HA, Santos AF, Makinson K, Penagos M, Stephens AC, Douiri A, et al. Peanut protein in household dust is related to household peanut consumption and is biologically active. J Allergy Clin Immunol 2013; 132:630-8.
11. Brough HA, Simpson A, Makinson K, Hankinson J, Brown S, Douiri A, et al. Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. J Allergy Clin Immunol 2014; 134:867-75.e1.
12. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol 2008; 122:984-91.
13. Bahnson HT, du Toit G, Lack G. Statistical Considerations of Food Allergy Prevention Studies. J Allergy Clin Immunol Pract 2017; 5:274-82.
14. Ierodiakonou D, Garcia-Larsen V, Logan A, Groome A, Cunha S, Chivinge J, et al. Timing of Allergenic Food Introduction to the Infant Diet and Risk of Allergic or Autoimmune Disease: A Systematic Review and Meta-analysis. Jama 2016; 316:1181-92.
15. Bellach J, Schwarz V, Ahrens B, Trendelenburg V, Aksunger O, Kalb B, et al. Randomized placebo-controlled trial of hen's egg consumption for primary prevention in infants. J Allergy Clin Immunol 2016.

16. Wei-Liang Tan J, Valerio C, Barnes EH, Turner PJ, Van Asperen PA, Kakakios AM, et al. A randomized trial of egg introduction from 4 months of age in infants at risk for egg allergy. J Allergy Clin Immunol 2016.
17. Palmer DJ, Sullivan TR, Gold MS, Prescott SL, Makrides M. Randomized controlled trial of early regular egg intake to prevent egg allergy. J Allergy Clin Immunol 2017; 139:1600-7.e2.
18. Palmer DJ, Metcalfe J, Makrides M, Gold MS, Quinn P, West CE, et al. Early regular egg exposure in infants with eczema: A randomized controlled trial. J Allergy Clin Immunol 2013; 132:387-92.e1.
19. Natsume O, Kabashima S, Nakazato J, Yamamoto-Hanada K, Narita M, Kondo M, et al. Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT): a randomised, double-blind, placebo-controlled trial. Lancet 2017; 389:276-86.
20. Du Toit G, Roberts G, Sayre PH, Plaut M, Bahnson HT, Mitchell H, et al. Identifying infants at high risk of peanut allergy: the Learning Early About Peanut Allergy (LEAP) screening study. J Allergy Clin Immunol 2013; 131:135-43.e1-12.
21. Santos AF, Douiri A, Becares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin Immunol 2014; 134:645-52.
22. Koplin JJ, Peters RL, Dharmage SC, Gurrin L, Tang MLK, Ponsonby AL, et al. Understanding the feasibility and implications of implementing early peanut introduction for prevention of peanut allergy. J Allergy Clin Immunol 2016; 138:1131-41.e2.
23. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001; 357:752-6.
24. Woodcock A, Lowe LA, Murray CS, Simpson BM, Pipis SD, Kissen P, et al. Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years. Am J Respir Crit Care Med 2004; 170:433-9.
25. du Toit G, Sayre PH, Roberts G, Lawson K, Sever ML, Bahnson HT, et al. The allergen-specificity of early peanut consumption and the impact on the development of allergic disease in the LEAP Study Cohort. J Allergy Clin Immunol 2017.

Name of Trial	**Country**	**Type**	**Population**	**Intervention group (hen's egg protein per week)**	**Control group**	**N**	**Intervention period (age in months)**	**Outcome assessed (age in months)**	**Primary outcome**	**Outcome in ITT (p value)**
Enquiring About Tolerance (**EAT**)	UK	RCT, open label	general population	cooked **whole HE** (4g)	HE avoidance until 6 months of age	1303	3-6	12-36	HE allergy (OFC)	**RR** 0.69 (95% CI 0.40-1.18) (p= 0.17)
Hens' Egg Allergy Prevention (**HEAP**)	Germany	RCT, blinded	general population	pasteurised **raw HE white** powder (7.5g) HE free diet	placebo powder (rice) HE free diet	298	4-12	12	HE sensitisation (sIgE)	**RR** 2.20 (95% CI 0.68-7.14) (p=0.24)
Solids Timing for Allergy Research (**STAR**)	Australia	RCT, blinded	high risk (infants with moderate/severe eczema)	pasteurised **raw whole HE** powder (6.3g)	placebo powder (rice)	86	0-8	12	raw HE allergy (OFC) and Sensitisation (SPT)	**RR** 0.65 (95% CI 0.38-1.11) (p=0 .11)
Starting Time for Egg Protein (**STEP**)	Australia	RCT, blinded	moderate risk (atopic mothers)	pasteurised **raw whole HE** powder (2.8g)	placebo powder (rice)	820	4-10	12	raw HE allergy (OFC) and Sensitisation (sIgE)	**Adj RR** 0.75 (95% CI 0.48-1.17 (p=0.20)
Beating Egg Allergy (**BEAT**)	Australia	RCT, blinded	moderate risk (1st degree relative with allergy)	pasteurised **raw whole HE** powder (2.45g) HE free diet	placebo powder (rice) HE free diet	254	4-8	12	HE sensitisation (SPT)	**OR** 0.46 (95% CI 0.22-0.95) (p=0.03)
Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (**PETIT**)	Japan	RCT, blinded	moderate risk (with atopic dermatitis)	heated **HE** powder (0.175g for 3 months then 0.875g for 3 months)	placebo powder (squash)	121	4-12	12	HE allergy (OFC)	**RR** 0·222 (95% CI 0·08–0·61) (p=0·0012)

Table 1. Summary of Randomized Controlled Trials with Hens Egg. ITT – Intention to treat. RR – Relative Risk. OR – Odds Ratio